

THE MATURATION OF RAT SKIN COLLAGEN IN VITRO

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The organization of the tropocollagen macromolecules into well-ordered aggregates in the connective tissue is an unique occurrence. The collagen matrix becomes resistant against dissolving forces with the advancing age and consequently less soluble into neutral salt solutions and acid buffers. The formation of the intra- and intermolecular linkages («cross-links») between peptide chains is thought to be the basis of that development.

To follow the maturation of collagen we injected L-proline-T (G) into newborn rats and after the incorporation period of 4 hrs the rats were killed and the skin slices incubated in different media. The rate of maturation was measured by determining the total hydroxyproline activity in both soluble and insoluble collagen fractions before and after the incubation (Heikkinen, E. *et al.*: *Acta physiol. scand.* Suppl. 277, 1966).

During the standard incubations of 6 hrs in glucose-containing Krebs-Ringer-phosphate medium the activity in the insoluble collagen fraction was doubled. The maturation was found to be affected by the following variables:

1. Temperature: At 0°C the maturation was negligible and rose with the temperature up to +37°C.
2. Slice size: In slices less than 0.3 mm thick the maturation was about 70% faster than in slices of 1.0 mm.
3. Calcium: In the absence of calciumions in the incubation medium the rate of maturation was lower than in corresponding standard incubations.
4. EDTA: EDTA in rising concentrations inhibited the rate of maturation.
5. »Carbonyl fixing» substances: For example phenylhydrazine in the concentration of 1.0 mM inhibited the maturation almost completely.

The maturation was not affected by the inhibition of the tricarboxylic acid cycle by sodium fluoride or of the glycolysis by iodoacetate. The inhibition of protein synthesis by puromycin did not have any effect on maturation.

The role of calciumions in the maturation and the mechanism of phenylhydrazine action are still obscure. The effect of phenylhydrazine gives further support to the idea that free carbonyl groups participate in the formation of »cross-links» in the collagenous structures.